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Effect of Formalin on the Viability of Pea Seeds

EFFECT OF FORMALIN
ON THE
VIABILITY OF PEA SEEDS

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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THE EFFECT OF FORMALIN ON THE VIABILITY OF PEA SEEDS.

I. INTRODUCTION.

Formalin is extensively used for disinfection purposes. In treating seeds with formalin for the destruction of parasitic fungi it has been found to exercise an injurious effect upon the germination of seeds and later development of seedlings. The present work was undertaken with the object of determining the effect of formalin treatment upon different varieties of peas.

In some instances the fungus is more resistant to the formalin treatment than the seed. In such cases some other than formalin treatment must be chosen. In every case it is well to know the greatest concentration of solution that can be applied without undue injury to the seed.

R. de Zeeuw (1911) experimenting with different agents as potassium dichromate, mercuric chloride, hydrogen peroxide, formalin, etc., concluded that it is possible to obtain only an antiseptic effect.

From the various investigators using this agent there come diverse statements concerning its effectiveness as a fungicide and its injury to seeds and seedlings.

In the prevention of smuts it has given different degrees of success. The dilutions used range from .02 to 2 per cent. The lower concentrations caused little injury to the seeds while the higher resulted in injuries sufficient to require great caution in its use.

Young (1919) states that formalin is a generally successful agent and that failure is usually due to lack of control on other factors.

The conflicting statements concerning its effectiveness show that more information concerning its relations towards many kinds of seeds is needed.

Since the treatment with formalin must necessarily be done in aqueous solution, the absorption of the chemical through the normal imbibition processes of the seed enters as an important factor in determining the degree of injury. The degree of permeability or impermeability and the resistance of the seed coat or protoplasmic membrane to the agent control the degree of vitality of the seed in treatment.

That there is a wide range in degree of permeability of the testa of different seeds is shown by the work of Crocker (1906), Brown (1907), (1909), and Shull (1913). Crocker found that such coats may be extremely impermeable to gases and liquids. Brown (1909) discovered that some seed coats may have a selective permeability and resist the action for example of sulphuric acid.

Shull (1913) found semi-permeability to be of wide occurrence in seed coats. He found that certain seeds can withstand, without injury, indefinite immersion in alcohol, ether and chloroform, providing the quantity of moisture present in the seed is very small. With some compounds the degree of injury is determined by the quantity of water present, save for those solutions of extreme dilution.

Denny (1917) found that substances responsible for the impermeability could be dissolved and the character of the membrane destroyed. He also found that temperature increased the permeability of the membrane. In studying the effect of different agents on the same kind of seeds it was found that there are wide differences in permeability and resistance exhibited among varieties.

In peas according to White (1917) this can be explained by differences in chemical composition, i. e., starchy or sugary peas. It was found that seeds with a larger sugar content and compound small-sized starch grains have a greater capacity for absorption of water. The opposite is true for the round, smooth

seeds which have large, simple starch grains and a smaller sugar content. Some of the latter seeds display greater impermeability of the coats and consequently have a greater resistance to the agent than have the peas with wrinkled coats and sugary content.

Miss Hind (1916) found that an increase in concentration decreased the time required to produce the toxic effect. Upon comparison of the organic with the inorganic acids used she observed that the organic compounds had a decidedly more rapid effect upon permeability. She thought that this was perhaps due to the anion as well as the cation, and the fact that proteins have a great absorbing power and ease of reacting with acids.

The activity of formalin and its power to combine with proteids has been offered as an explanation of its poisonous qualities. The conflicting results obtained from different investigations have given rise to a question concerning its toxicity in different concentrations.

Arcichovskij (1913) in his experiments with different concentrations of poisonous substances upon peas explained his results upon the basis of less ionization and greater polymerization in concentrated solutions. For the inorganic substances he suggests that they are due to the less relative and absolute ionization of concentrated as compared with that of the dilute solution. Although the proportion of ionized substances may be greater in the dilute, the numerical value for the concentration of the ions is less than the numerical value for the concentration of ions in the strong solutions. From this it would seem that the more concentrated solutions should be the most toxic. But this effect is greatly influenced by the permeability and resistance which the seed has for the particular compound or ions. The extent of these two qualities governs the penetration of the solution.

With the non-electrolytic substances he suggested that there was a greater polymerization in the concentrated solutions and so less activity.

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Bliss and Novy (1899) working with formalin found that the more concentrated solutions had a more toxic effect and that higher temperatures, as 40° C, increased the poisonous effect.

Sherman (1905) states that "polymeric modifications of formalin in aqueous solutions closely resemble the original substance in behavior to reagents".

On heating, the formalin that has polymerized breaks up to give formalin again. This is a characteristic for those substances which polymerize.

The following experiment was performed to determine the effect of formalin upon the germination of different varieties of peas. Since different concentrations, as weak and strong often have unlike effects, or a similar effect but in different degrees, several concentrations were used. In order to observe the relation which time has to concentration different periods of soaking were carried out.

II. MATERIALS AND METHODS.

Four varieties of peas; Alaska, Canada Field, Black-eyed Marrowfat and Gradus were used. These varieties give a wide range in the degree of permeability of the coats as well as a marked difference in chemical composition, particularly with regard to sugar and starch content.

Formalin of varying concentrations closely following those of Arcichovskij's experiment were used. The actual soaking was carried on at a constant temperature of 35° C. The seeds were selected to eliminate those with broken coats or other imperfections. Fifty weighed seed of each variety were placed in glass-stoppered bottles and together with the prepared solution subjected for five hours to the constant temperature of 35° C. At the end of this period the solution was poured over the seeds. They remained in the formalin for thirty, sixty or ninety minutes. At the end of each soaking the solution was poured off, and the seeds thoroughly rinsed in tap water. They were then superficially dried between towels.

Since the time of immersion largely determines the absorption of water and the chemical in solution the seeds were again weighed after treatment.

After weighing, the seeds were placed on plaster of paris blocks in germinating pans at 22° C. for germination.

A period of eleven days was taken as the maximum to allow for germination. In a few cases where seeds looked as if they had still the power to germinate more time was given. In order to count a seed as germinated the tip of the root or shoot had to be free from the coat.

Each concentration was repeated three times. At the end of each experiment the blocks and pans were carefully washed and allowed to dry over night.

III. RESULTS AND DISCUSSION.

For the four varieties a general similarity of results was obtained. The graphs showing this are based on the averages of the three repetitions of each concentration, the numerical values of which are given in tables I, II, III and IV.

For the curves showing increase in weight there is a rise from one per cent to 16 percent, at which point the greatest absorption occurs, succeeded by a more or less gradual decrease.

For Alaska, as shown by the curves in figurel, the rate of absorption in solutions of different concentrations, for periods of 30 and 60 minutes, shows a sharp decline to the 3 percent solution at which concentration there is a rapid rise. Seeds having a 90 minute immersion show this rise starting at 2 percent. This is perhaps due to a certain degree of impermeability and resistance which the seed coat of Alaska may have for formalin. In the longer periods of treatment as 90 minutes a sufficient time elapses to allow a dilute solution to affect this partial impermeability. That there should be a drop at all may be explained by the behavior of the water molecules in the imbibitional process. The seed coat with its varied degree of impermeability and resistance prevents the entrance of large quantities of the solute. The formalin may oppose to some extent the attraction of the seed coat for water and so result in a smaller increase in weight or a decrease in the rate of absorption.

The greatest absorption occurs in a 16 percent solution because the proportion between solute and solvent is most favorable. The decrease in weight gain for formalin (40 percent formaldehyde) and a 32 percent solution may be due in part

to an exosmosis during treatment as well as to the smaller amount of available water in the stronger concentrations.

There is a close correlation between the curves for germination and the corresponding curves for rate and quantity of absorption. A slight increase in absorption shows a corresponding decrease on the germination curve, for instance the two curves between the concentrations 3 and 4 percent for seeds treated 30 and 60 minutes.(Fig. 1). The germination for Alaska is greatly affected by increase in concentration until a 16 percent solution is reached; a 32 percent solution shows a decided rise which may be due to the decreased water content limiting the quantity of formalin and the depth of penetration and so the injury. The drop in germination which a 40 percent solution occasions is perhaps due to the increased amount of formalin overcoming the effect of a decreased water content.

Canada Field presents some differences. There is a smaller absorption of the solution in each of the concentrations, which is in keeping with its smaller size and higher degree of impermeability of its coats for water in comparison with Alaska or Gradus. The impermeability or slow permeability of its coats for solutions of the higher concentrations acts as a protection and explains the greater toxicity of the dilute solutions, as 4 percent. The curves for Canada Field illustrate well that the smaller the absorption the less is the degree of injury. (Figure 2).

Of the four varities the coats of Gradus are the most permeable to water. As with Alaska the greatest absorption occurs in a 16 percent solution. (Figure 3). The curve for a 60 minute treatment exhibits a decline at 2 percent concentration and then a rise at 3 percent. For a 90 minute immersion there is an abrupt rise to the 3 percent solution followed by a decrease for 4 percent, from which point occurs a more gradual rise to 16 percent. The rate of absorption is not

uniform for a whole period. This may account for the changes in weight for the concentrations from 1 to 4 percent. The curve (Figure 3) for a 60 minute immersion in a 2 percent solution shows the toxic action is still too weak to affect the permeability which offers a certain opposition to the absorption of the solution. With the increase to 3 percent an increased injury results and permits an increase in absorption over the 2 percent solution. The longer period of soaking brings out the differences that concentrations may show, particularly where dilute solutions are used.

Black-eyed Marrowfat was treated only with concentrations through 8 percent. There is a decrease in rate of absorption from 2 percent to 8 percent solutions which results from the decrease in water content and the limiting effect which time has upon concentrations. The curve for a 60 minute immersion more clearly shows this. (Figure 4).

Comparing the absorption curves for the four varieties the decrease for the high concentrations which Gradus and Canada Field present is caused by the diminished water content of the solution and in the case of Gradus an increased exosmosis during treatment. This decrease is not evident for Alaska seeds soaked for 60 and 90-minute periods as will be seen upon comparison of the total absorption at 40 percent and 3 percent respectively. (Figure 1).

Canada Field because of the large number of impermeable coats and differences in permeability of the successive random samples shows a higher germination for seeds soaked 90 minutes than those for 60 minutes when a 40 percent solution was used. With this exception the longer the period of soaking the greater is the degree of injury to the seeds as shown by the percent of germination.

The relation between time and concentration is shown by the similar effects upon germination which a high concentration acting for a thirty minute period exerts as compared with a low concentration for 60 or 90 minute treatment. This is

shown in Figure 1 where Alaska treated for 30 minutes with formalin (40 percent formaldehyde) solution gives a result approximately equal to a 60 minute treatment with 4 percent.

The belief that the high concentrations have a much smaller poisonous effect than weaker concentrations is not supported for formalin by these experiments.

The injury for the strongest solutions is approximately that for 2, 3 and 4 percent solutions, the closeness depending upon the period of soaking. Both concentrations exert a marked injury. Even for a 1 percent solution there was a slight injury.

The occurrence and development of fungi as Rhizopus, Penicillium and Aspergillus were not the same for all the varieties. The growth of fungi on treated peas results from the diffusion from the seeds injured by the treatment. The growth of the mold was closely related to the degree of absorption of water and solute.

For the dilute solutions, 1 and 2 percent there was less mold and its occurrence was invariably upon those seeds which had highly permeable or broken coats.

Canada Field had very little mold until a 4 percent solution was used. From this concentration on there was always a fairly evident development of white mold by the third day. Four or five days had been required in the weaker solutions for a fairly evident growth. The white mold developed best where there was seemingly a slight or moderate diffusion of organic materials from the seed.

Similarly the variety Alaska had only a slight development of fungi for concentrations 1 and 2 percent. With a 3 percent solution seeds treated for 90 minutes sometimes had no mold. When 4 and 8 percent solutions were used the amount of Penicillium and Aspergillus was increased while that of Rhizopus was

inhibited.

With the use of higher concentrations the exosmosis was more pronounced and the quantity of formalin retained by the seed tissues probably much increased. This may account for the slight growth of Penicillium on seeds treated 60 and 90 minutes. Treatment with a 40 percent solution resulted in a decreased germination and comparatively little mold.

Gradus showed similar conditions for the dilute solutions 1 and 2 percent Seeds treated with 8 percent formalin for 30 and 60 minutes showed in some instances white mold well started within four or five days. But those treated for 90 minutes were free from Rhizopus. Penicillium developed in some cases after five or six days, though usually there was no growth. For 16 and 32 percent solutions Gradus showed the largest amount of exosmosis of the four varieties. The seeds immersed 60 or 90 minutes were most affected and had little or no mold development.

Mold occurred upon the checks but never to the extent that it did upon treated seeds. There was only very slight development of Penicilluim and Aspergillus upon the checks; the most common and abundant fungus were species of Rhizopus.

The vigor of germination was markedly affected by all concentrations above 2 percent.

The seeds subjected to a 30 minute immersion in a 1 percent solution displayed a vigorous growth, but with longer periods of treatment injury became apparent. Some seeds, when 2 and 3 percent solutions were used, made a fair growth but many exhibited a very weak germination and usually soon died.

Of the four varieties Canada Field suffered the most. With higher concentrations its root just broke the coat, coiling up beneath.

IV. CONCLUSION

From a consideration of the results presented, formalin in solutions as dilute as one percent exerts an injurious effect, as shown by decreased germination, and a decreased vigor of seedling growth.

The poisonous effect of the higher concentrations was greater than that of the dilute for a thirty minute period of soaking in the case of Alaska and Gradus.

Longer periods of soaking tend in the case of Alaska and Gradus to give equal degrees of toxicity for high and low concentrations.

The smaller degree of injury upon Canada Field results from the nature of its coats which have a higher degree of impermeability to water.

The greatest injury is caused by solutions of medium concentrations.

Table I. Alaska.

Length of Treatment		Concentrations. 1	2	3	4	8	16	32	40
30 Minutes	Percent weight increase	13	12	9	16	25	29	28	24
	Percent germination	97	92	92	66	40	34	43	23
60 Minutes	Percent weight increase	33	30	25	34	41	42	36	31
	Percent germination	85	61	55	19	13	2	23	13
90 Minutes	Percent weight increase	47	42	43	47	49	57	45	36
	Percent germination	51	17	4	4	2/3	2/3	11	7
Check, percent germination		99	100	99	99	100	100	99	99

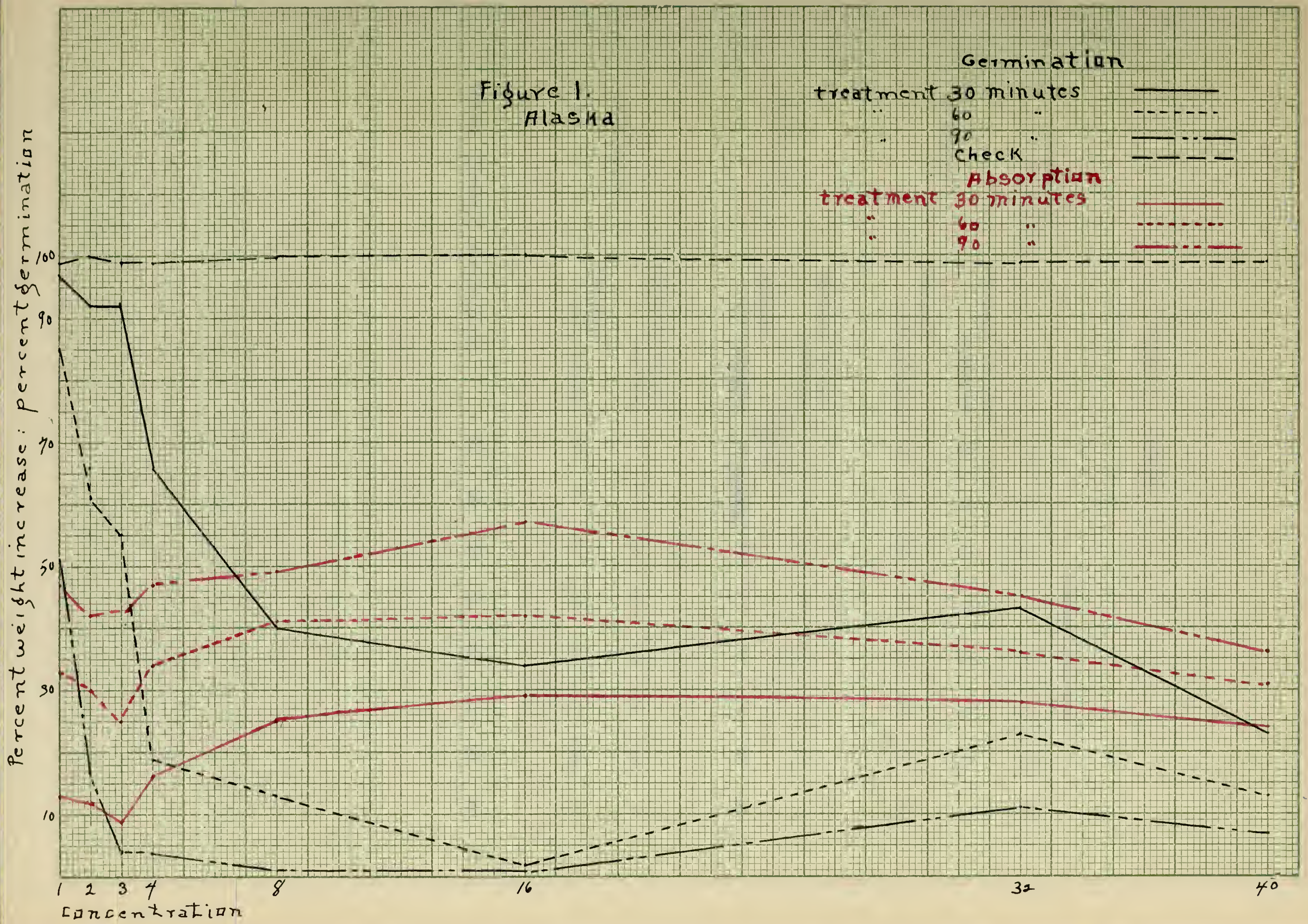
Figure 1.
Alaska
Germination
treatment 30 minutes
" 60 "
" 90 "
Check
Absorption
treatment 30 minutes
" 60 "
" 90 "
100
90
70
50
30
10
1 2 3 4 8 16 32 40
Concentration
Percent weight increase: percent germination

Table II. Canada

Length of Treatment		Concentrations 1	2	3	4	8	16	32	40
30 Minutes	Percent weight increase	8	6	8	7	8	7	5	1
	Percent germination	86	83	55	53	50	57	65	56
60 Minutes	Percent weight increase	19	17	14	20	13	17	10	4
	Percent germination	64	49	46	23	38	34	37	40
90 Minutes	Percent weight increase	27	26	24	25	23	25	13	2
	Percent germination	50	41	27	16	25	27	34	43
Check, percent germination		92	95	92	91	91	91	85	93

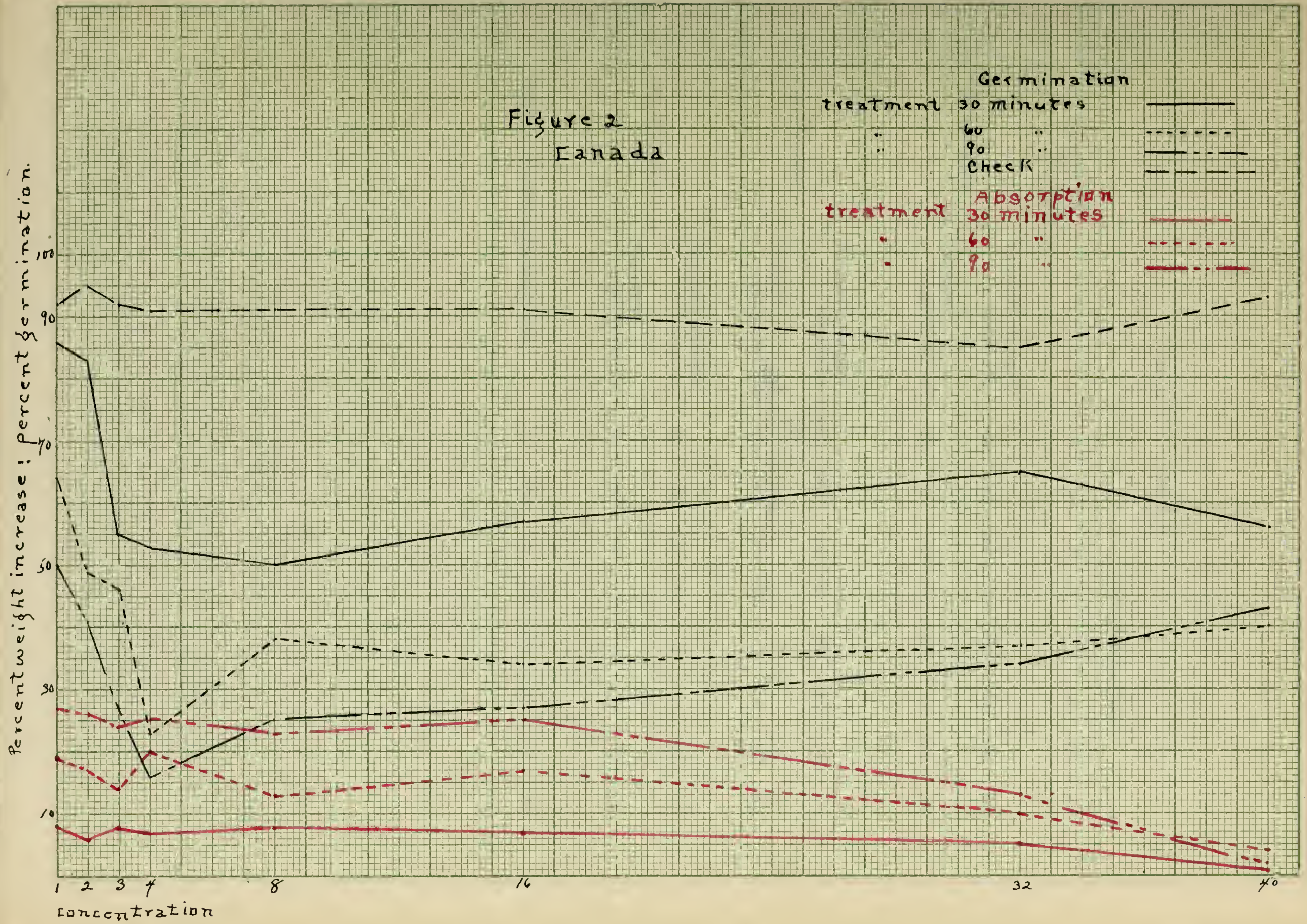
Figure 2
Canada
Germination
treatment 30 minutes
" 60 "
" 90 "
Check
Absorption
treatment 30 minutes
" 60 "
" 90 "
Percent weight increase; percent germination.
100
90
70
50
30
10
1
2
3
4
8
16
32
40
concentration

Table III. Gradus.

Length of Treatment		Concentrations 1	2	3	4	8	16	32	40
30 Minutes	Percent weight increase	33	34	33	33	38	41	31	20
	Percent germination	75	56	46	38	18	16	24	25
60 Minutes	Percent weight increase	52	45	54	50	48	53	37	19
	Percent germination	55	37	17	10	9	1	17	21
90 Minutes	Percent weight increase	55	58	61	54	61	64	42	21
	Percent germination	51	15	9	3	4	2	5	16
Check, percent germination		93	98	91	83	83	87	91	83

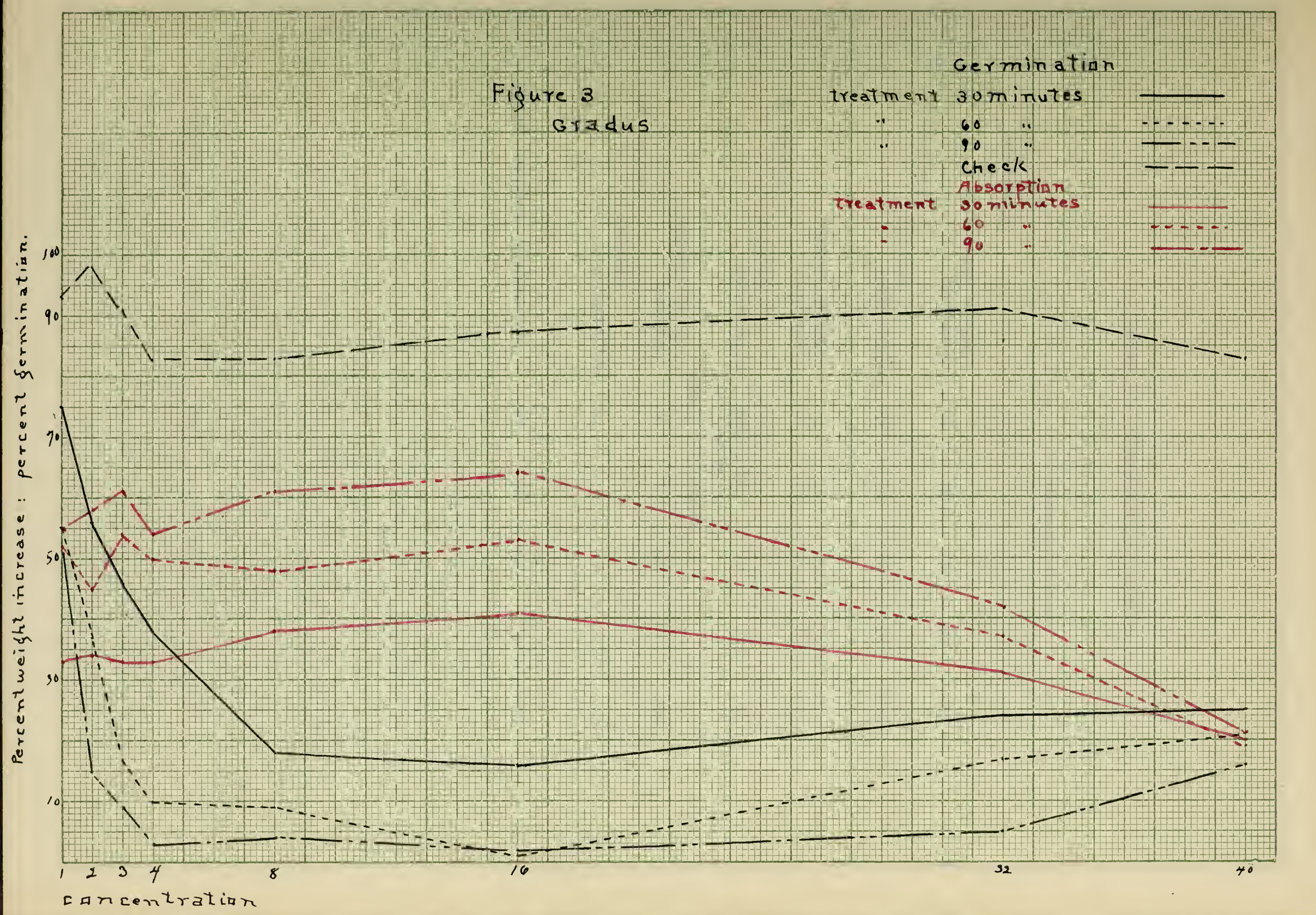

Figure 3
Gradus
Germination
treatment 30 minutes
" 60 "
" 90 "
Check
Absorption
treatment 30 minutes
" 60 "
" 90 "
100
90
70
50
30
10
1 2 3 4 8 16 32 40
concentration
Percent weight increase : percent germination.

Table IV. Black-eyed Marrowfat.

Length of Treatment		Concentrations 1	2	3	4	8
30 Minutes	Percent weight increase	17	18	15	14	14
	Percent germination	64	48	39	28	26
60 Minutes	Percent weight increase	34	34	31	30	26
	Percent germination	36	21	19	9	15
90 Minutes	Percent weight increase	42	43	42	41	43
	Percent germination	21	8	7	2	7
Check, percent germination		94	96	81	76	76

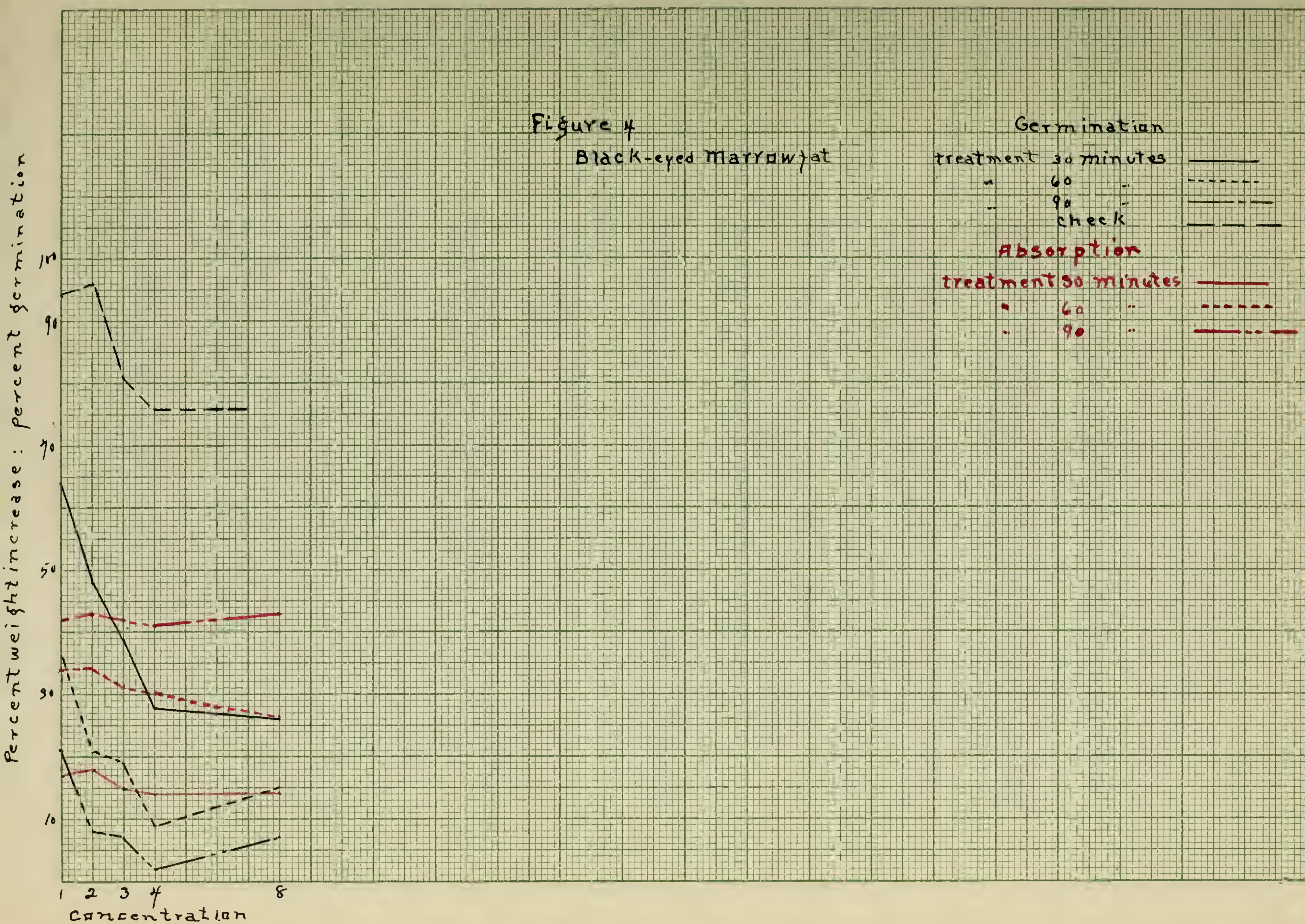
Figure 4
Black-eyed Marrowfat
Germination
treatment 30 minutes
" 60 "
" 90 "
check
Absorption
treatment 30 minutes
" 60 "
" 90 "
Percent weight increase : percent germination
100
90
70
50
30
10
1 2 3 4 8
Concentration

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